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PATENT

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Applicant:	McKeown, et al.	Examiner:	WHALEY, Pablo S.
Serial No.:	10/632,393	Group Art Unit:	1631
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December 15, 2006

Commissioner for Patents
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AMENDMENT AND REPLY TO OFFICE ACTION

Sir:

This amendment is a reply to the Final Office Action mailed on August 28, 2006 in connection with the above-identified application. The time set for filing a response to the Office Action expired on November 28, 2006. Applicants submit a petition for one-month extension of time and enclose a check for the associated fee. Accordingly, the time for responding to the Office Action now expires on December 28, 2006 and this amendment is timely filed.

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An advantage of the invention is that it allows the accurate genotyping of template DNA samples in which there are greater than two haplotypes of a particular form, or an apparent asymmetry in the number of copies of a particular haplotypes present in a target DNA sample. This may be observed as, for example, additional copies of the PrP (prion protein) locus in sheep, or as an asymmetric mixture of two or more template DNAs that results in an apparent asymmetry of a specific variant nucleotide, or cluster of variant nucleotides identified as a specific haplotype. The invention can be applied to a controlled breeding program. The presence of more than two haplotypes of a particular form can complicate breeding strategies to increase or decrease the prevalence of specific haplotypes. A further advantage of the invention is that it enables the quantification of the relative abundance of specific variant nucleotides in a target DNA sample, and interpretation of the ratio of each variant nucleotide.

The figures have been simplified for clarity. For example, the extension product of a primer that flanks a variant nucleotide is shown as a single peak in the figures, as would be the case if the variant position were homozygous. If the variant position was heterozygous, two very closely associated peaks may be generated, with the two extension products having very slightly different mass:charge ratios, due to the different terminal base incorporated, and possibly the different labels attached to the terminating base. Differences in 5' tags can alter mass:charge ratios.

As employed herein, "S" refers to a G or a C, "R" refers to an A or a G, "Y" refers to a T or a C, "K" refers to a G or a T and "M" refers to a C or an A.

Figure 1 illustrates an actual genotyping profile of a sheep analyzed at the PrP locus. The profile was generated using an assay disclosed in US Patent Application Serial No. 10/179,826, NOW ABANDONED. This profile shows unexpected imbalance at the heterozygote positions labeled with arrows 1 (solid black) and 2 (diagonal stripes), where the expected pattern would have the peak indicated by arrow 2 marginally larger than that of arrow 1. It is very much smaller in this example. Those peaks indicated by arrows 3 (white) and 4 (horizontal stripes) are also unexpectedly imbalanced, with the peak indicated by arrow 4 normally being marginally larger than that indicated by arrow 3. In this example it is significantly larger.

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with desirable characteristics, including but not limited to microinjection of genomic material into germ cells and embryos.

Genotyping of sheep with respect to the PrP locus, with the knowledge of multi-allelic PrP genes, and with selective breeding of individual sheep selected for the presence of desirable genotypes, can lead to a scrapie-resistant sheep population.

More than 650,000 individual animals have been genotyped by methods of the present invention, relying on a proprietary primer extension assay, SNP-IT™, which interrogates the four SNPs that determine the coding potential at positions 136, 154 and 171 (codon 171 harbors two polymorphic nucleotides, and can encode arginine, glutamine or histidine).

In one embodiment, the assay employed here relies on the generation of a single 310 bp amplicon of the scrapie gene that contains all four of the polymorphic nucleotides. This amplicon then serves as the template during a multiplexed fluorescent primer extension assay. As the extension primers employed in this assay are distinct in both size and sequence, they can be separated on a capillary electrophoresis apparatus to enable the bases present at the polymorphic sites and therefore infer the amino acids which will be present in the protein. A preferred assay suitable for use with the present invention is disclosed in US Patent Application Serial No. 10/179,826, NOW ABANDONED filed 25 June 2002, the entire disclosure of which is hereby incorporated by reference.

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The profiles generated by the assay developed here have become familiar and predictable in terms of peak intensity, both between different SNPs, and more particularly, between the different peaks of a heterozygous call. Clearly aberrant profiles were initially observed, and initially appeared to be due to a secondary contaminating template being present during the initial PCR reaction. However, repeat analysis and retesting from fresh samples taken from a separate bleed of the interrogated animals established that the imbalances are real, and due to the occurrence of complex genotypes in the individuals exhibiting the imbalances.